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Full Length Research Paper

Hazards analysis critical control points (HACCP) and microbiology qualities of sea-foods as affected by handler's hygience in Ibadan and Lagos, Nigeria

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This study reports the hazard analysis critical control points (HACCP) and microbiological qualities of seafood products as affected by hygiene of handlers in Ibadan and Lagos, Nigeria. Seafood products were purchased at four different processing plants, two each from Ibadan and Lagos. This study consisted of observing the raw materials, water used, the utensils used and the environment, monitoring all steps of the processing and packaging, recording temperatures during preparation, storage and display and collecting samples of seafood products for total viable counts, coliforms, *Salmonella* and *Shigella* counts and fungi counts. A microbiological survey of seafood processors/handlers was also performed. Palms of processors/handlers were swabbed and analyzed for the presence of indicator organisms of sanitary quality. The total viable bacteria count decreased with time from raw to packaging and to when ready for consumption. Coliforms, *B. cereus*, *Staphylococcus aureus*, *Salmonella* sp, and fungi were isolated and had count higher than 10^3 cells/ml before processing and after processing with few exceptions. A total of 186 organisms were isolated and identified. Almost all palms of the handlers sampled haboured *Micrococcus* sp. [36(21.1%)] and *Enterobacter* sp. [30(18.0%)], followed by *Bacillus* sp. [24 (14.0%)], *Flavobacterium* sp. [19 (11.1%)], *Staphylococcus* sp. [17(9.9%)], *Escherichia coli* [15(8.8%)], *Proteus sp* [8(4.7%)], *Salmonella* sp. [7(4.1%)], *Citrobacter sp* [3(1.8%)], *Klebsiella sp* [4(2.3%)], *Pseudomonas sp.* [3(1.8%)], *Serratia sp.* [3(1.8%)] and *Achromobacterium sp* [2(1.2%)], *Aspergillus formigatus* [3(20.0%)], *Aspergillus niger* [2(13.3%)], *Fusarium sp* [2(13.3%)], *Mucor mucido* [3(20.0%)], *Neurospora crassa* [2(13.3%)] and *Rhizopus* sp [3(20.0%)]. Seafood processors/handlers may be sources of microbial chance inoculation, microbial food poison, food intoxication and food spoilage hence, processors/handlers may be counter productive by being responsible for public health hazard and loss of revenue. The level of counts appears high for processed and unprocessed products; the presence of coliforms, *S. aureus*, *B. Cereus*, *Salmonella* and fungi showed that processing of these seafood products in a highly contaminated environment and holding at frozen temperature for sale could be risky. Using current WHO/FAO/NAFDAC guidelines and standards for foods and water, none of the food processors/handlers, the utensils and the products were within acceptable standards. There were significant correlations between bacteriological quality and food hygiene training, and waste product management polices. The findings of this study suggests that there is need to improve on hygienic practices as well as HACCP implementation in public food service outlets in order to obtain relatively safe processed seafood products for consumption. The new approach to supervision of food hygiene and sanitary quality, the HACCP system works rationally as it is based on analysis of systematically assembled data on the causes and conditions which evoked the illness of the consumers by food pro-

ducts or meals. Therefore, education of owners of seafood processing plants, processors/handlers on hazards, critical control points and the importance of hygienic environment is imperative. The control measures and monitoring procedures for seafood processing and packaging are thus advocated.

Key words: Coliforms, contamination, frozen seafood, hazards, food hygiene, CCP, control measures, monitoring, microbiological quality, sanitary standards.

INTRODUCTION

The term "fish" includes all fresh or saltwater finfish, molluscan shellfish, crustaceans, and other forms of aquatic animal life. Fish and shellfish are an important part of a healthful diet. They contain high quality protein and other essential nutrients can be low in saturated fat and may contain omega-3 fatty acids. In fact, a well-balanced diet that includes a variety of fish and shellfish can contribute to heart health and children's growth and development and safety but, as with any type of food, it's important to handle seafood safely in order to reduce the risk of foodborne illness. Follow these basic food safety tips for buying, preparing, and storing fish and shellfish - and you and your family can safely enjoy the fine taste and good nutrition of seafood.

Fish products have an essential role in the traditional European diet due to their composition and the high number of fish species. Fish products contribute signify-cantly to “healthy diets” due to their high content on w-3 polyunsaturated fatty acids (w-3 PUFA), and other important components as high quality proteins, vitamins or minerals . However, fish products are very prone to degradation (Liston, 1982). Their high water content, autochthon bacteria flora able to live at low temperatures and its high enzymatic activity, mainly autolitic, are responsible of the susceptibility of fish muscle. Among these changes, lipid oxidation is one of the most important. It leads to rancid flavours and reduces the shelf–life of fish products especially during storage (Flick and Martin, 1992).

Fish containing bioactive lipids are an excellent source of polyunsaturated fatty acids and its consumption has been related with the prevention of cardiovascular and other diseases (Medina et al., 2008). However, oxidation of PUFA leads to the development of off-flavours and rancidity and continues to be the main objection in their pr-duction and commercialisation. Natural phenolic antioxidants have been proposed as inhibitors of lipid oxidetion in these food products (Medina et al., 2008).

Seafood products consisting of peeled shrimps, headless shrimps, jumbo prawn, croaker filets, sole filets, fish steaks, calamari cleaned, red mullets, lobster and crab, fish fingers, seafood mix, and seafood skewers, packed in take-away packs and polythene bags and sold at frozen temperature are becoming popular in Nigeria markets. Mainly washing procedures, processing and storage temperature influences the shelf-life of these products. The short shelf-life of one month for processed seafood products, the unhygienic condition of local processing, pitching with mixed culture from fresh raw frozen seafood products, and the resultant poor packageing; inconsistent power supply and lack of decent environment for different processors are limitation to safe processed seafood products.

The presence of large numbers of *B. cereus* (greater than 10^6 organisms/g) in a food is indicative of active growth and proliferation of the organism and is consistent with a potential hazard to health (Walderhaug, 1992). Contamination of these seafoods is usually through the fecal-oral route. Fecally contaminated water and unsanitary handling by food handlers are the most common causes of contamination (Walderhaug, 1992, 2001, 2008). Treatment of produce with chlorinated water reduces populations of pathogenic and other microorganisms on fresh produce but cannot eliminate them. Reduction of risk for human illness associated with raw produce can be better achieved through controlling points of potential contamination in the field; during harvesting; during processing or distribution; or in retail markets, food-service facilities, or the home (PHPP, 1997).

In Nigeria, there is a large number of public frozen seafood processing services distributed along the country, where a considerable number of people buy their frozen seafood product daily. Processing of seafood products are usually conducted in small scale or cottage industries thus it entirely depends on spontaneous preparations in such outlets and is without implementation of either Good Manufacturing Practices (GMPs) or Hazard Analysis and Critical Control Point (HACCP) systems. This is considerably very risky (Nout and Motarjemi, 1997; Kopermsub and Yunchalard, 2008).

USFDA (2007) recommends that seafood be purchased only from reputable sources. These recommendations include: be wary, for example, of vendors selling fish out of the back of their pick-up trucks. Buy only fresh seafood that is refrigerated or properly iced. Do not buy cooked seafood, such as shrimp, crabs or smoked fish if displayed in the same case as raw fish. Cross-contamination can occur. Do not buy frozen seafood if the packages are open, torn or crushed on the edges. Avoid packages that are above the frost line in the store's freezer. If the package cover is transparent, look for signs of frost or ice crystals. This could mean that the fish has either been stored for a

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a long time or thawed and refrozen. Put seafood on ice, in the refrigerator or in the freezer, immediately after buying it. Recreational fishers who plan to eat their catch should follow state and local government advisories about fishing areas and eating fish from certain areas. (USFDA, 2007)

Serious consequences relating to national productivity and development can arise from lack of hygiene and sanitation in such outlets. Seafood products are usually sold at frozen temperature and are held that temperature till supplies were exhausted. Leftovers were frozen at -20^{O}C. There are many concerns about the sanitation of shop-vended seafood products. For example, well water is the main source of water in many localities in Ibadan and the part of Lagos under study; all the wells and shops are situated near the Lagoon; the area is highly water logged and characterized with lack of sewage systems; the raw seafood products, water and the utensils used are highly prone to contamination (Inabo et al., 2000), and processing and packaging are done mainly by uneducated workers living around these areas with poor sanitary conditions (Oranusi et al., 2003).

There have been several reports on the health risks associated with the consumption of processed seafood, ranging from allergic reactions, stomach and intestinal cancerous growths, a general degeneration of peripheral cellular tissues, to gradual breakdown of the digestive and excretive systems in a statistically high percentage of people examined (Edema et al., 2005). Few of these reports however, have looked at the likely risks from a microbiological food safety point of view (Edema et al., 2005).

The potential of water to harbour microbial pathogens and causing subsequent illness is well documented for both developed and developing countries (Younes and Bartram, 2001; Wright et al., 2004). Water-related diseases continue to be one of the major health problems globally (UNESCO, 2003). It is estimated that 80% of all illnesses are linked to use of water of poor microbiological quality (WHO, 2002). One of the strategies for tackling this problem is the provision of protected sources such as boreholes, standpipes, protected wells and springs (Ahmed et al., 1998). However, such facilities are located some distances requiring transportation to homes (Taulo et al., 2008). During transportation, water gets contaminated with bacteria which grow and proliferate during storage in the homes (Hoque et al., 2006). This contamination may lessen the health benefits of water source improvements (Wright et al., 2004).

The microbiological quality of seafood products and water used in processing them is achieved by as far as possible ensuring the absence of pathogenic microorganisms and by all means preventing their multiplication (Edema and Omemu, 2004; Okonko et al., 2008b,c). To develop a better understanding of the microbiological problems associated with seafood processing and packaging, it became extremely necessary to apply the hazard analysis critical control point (HACCP) strategy. HACCP strategy identifies hazards associated with different stages of processing, packaging and handling, assesses the relative risk and identifies points where to control measures would be effective (Bryan, 1988; Ehiri et al., 2001; Oranusi et al., 2003).

Hazard Analysis and Critical Control Points (HACCP) is a systematic preventive approach to food safety and pharmaceutical safety that addresses physical, chemical, and biological hazards as a means of prevention rather than finished product inspection. HACCP is used in the food industry to identify potential food safety hazards, so that key actions, known as Critical Control Points (CCP's) can be taken to reduce or eliminate the risk of the hazards being realized. The system is used at all stages of food production and preparation processes including packaging, distribution, etc. The Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA) use mandatory juice, seafood, meat and poultry HACCP programs as an effective approach to food safety and protecting public health. Meat and poultry HACCP systems are regulated by the USDA, while seafood and juice are regulated by the FDA. The use of HACCP is currently voluntary in other food industries (FSIS, 2007; USFDA, 2007; FSRIO, 2008).

HACCP was conceived in the 1960s when the US National Aeronautics and Space Administration (NASA) asked Pillsbury to design and manufacture the first foods for space flights. Since then, HACCP has been recognized internationally as a logical tool for adapting traditional inspection methods to a modern, science-based, food safety system. Based on risk-assessment, HACCP plans allow both industry and government to allocate their resources efficiently in establishing and auditing safe food production practices. In 1994, the organization of *International HACCP Alliance* was established initially for the US meat and poultry industries to assist them with implementing HACCP and now its membership has been spread over other professional/industrial areas (IHA, 2007).

In the Federal Register of December 18, 1995, FDA published the seafood HACCP (Hazard Analysis and Critical Control Point) regulation (60 FR 65096). The seafood HACCP regulation requires seafood processors to conduct an analysis of the potential food safety ha-zards that are reasonably likely to occur with the seafood products they process and to have and implement written HACCP plans to control any hazards identified in the hazard analysis. FDA has published three editions of the Fish and Fisheries Products Hazards and Controls Guidance (the Guide) as assistance to the seafood processing industry in developing seafood HACCP programs. The Guide covers food safety hazards that are associated with fish and fishery products and provides examples of recommended preventive measures to minimize the likelihood of a hazard's occurrence.

The primary purpose of US Food and Drug Administra-

tion (USFDA) guidance is to assist processors of fish and fishery products in the development of their HACCP plans. Processors of fish and fishery products will find information in this guidance that will help them identify hazards that are associated with their products, and help them formulate control strategies. Another purpose of this guidance is to help consumers and the public generally to understand commercial seafood safety in terms of hazards and their controls. This guidance does not specifically address safe handling practices by consumers or by retail establishments, although many of the concepts contained in this guidance are applicable to both. This guidance is also intended to serve as a tool to be used by federal and State regulatory officials in the evaluation of HACCP plans for fish and fishery products (USDA, 2007; FSRIO, 2008).

Hence, HACCP has been increasingly applied to Industries other than food, such as cosmetics and pharmaceuticals. This method, which in effect seeks to plan out unsafe practices, differs from traditional "produce and test" quality assurance methods which are less successful and inappropriate for highly perishable foods. In the US, HACCP compliance is regulated by 21 CFR part 120 and 123. Similarly, FAO/WHO published a guideline for all governments to handle the issue in small and less developed food businesses (FAO/WHO, 2007).

Seafood can be exposed to a range of hazards from the water to the table. Some of these hazards are natural to seafood's environment; others are introduced by humans. The hazards can involve bacteria, viruses, parasites, natural toxins, and chemical contaminants. The HACCP system that seafood companies will have to follow will help weed out seafood hazards (USFDA, 2007).

The Hazard Analysis Critical Control Point (HACCP) concept is used to identify microbiological vulnerable points in the food production process and processing, to determine the most appropriate methods of control to be applied, usually such methods as improved handling techniques, monitoring of temperature and more intensive supervision (Edema and Omemu, 2004). This study therefore, assesses the hazards associated with consumption of seafood products and identifies the critical control points (CCP) and sought to evaluate the microbiological quality of processed seafood products. This study also aimed at evaluate the incidence of biological pathogens particularly microbes in our processed frozen seafood products, with a view to providing potential approaches to improve their quality, consumer safety and sanitary standard for the processing plants.

Therefore, this current study reports hazard analysis critical control points (HACCP) and microbiological quailties of seafood products as affected by hygiene of handlers in Ibadan and Lagos, Nigeria. Physicochemical and microbiological quality of the water samples used in processing these products was also evaluated. Measures that could ensure safety of seafoods are emphasized. This study will help to develop a better understanding of the microbiological problems associated with processing seafood products; it became extremely necessary to apply the hazard analysis critical control point (HACCP) strategy. HACCP strategy identifies hazards associated with different stages of preparation and handling, assesses the relative risk and identifies points where control measures would be effective (Bryan, 1988; Ehiri et al., 2001; Oranusi et al., 2003).

MATERIALS AND METHODS

Selection of processing plants

This was carried out as described by Oranusi et al. (2003). Preceding the hazard analysis was a survey study of 60 samples seafood products processed, packed, frozen and bulk-packaged seafood purchased from different processing plants and handlers in Ibadan and Lagos, Nigeria. The study resulted in a close interaction with the owners of processing plants, processors/handlers. Based on the packaging method, location, type and number of consuming population and willingness to participate in the study, four processing plants were selected, two each from Liberty road, Ibadan; and Ijora-Olopa, Lagos City for hazard analysis and evaluation of microbiological quality.

Description of processing plants, the processors/handlers and vending operations

Processing plant A belongs to very industrious lady who lived in a three-bed room flat at Surulere, Lagos State and has her plant situated in Ijora-Olopa, Lagos Island, Lagos State. Water used for processing was fetched from uncovered wells situated in-front of the plants and stored in drums and uncovered large plastic containers. The owner of the processing plants keeps snails and has staff strength of 7 members. All her family members are also involved in the seafood business. The finished products was sprayed in trays and stored at commercial cold room to be frozen. This was later weighed and packaged in take-away disposable packs and polyvinyl chloride (PVC) packs, sealed; stored in freezers and sold in their shopping marts. The plants supplies their products to major shopping marts, supermarkets and hotels in Ibadan and Lagos. The unsold product from these major shop dealers were returned back after a long period or if there were too many complains from the customers. These products were either re-processed and repackaged for another supply or discarded if it has deteriorated.

Processing plant B also belong to the lady above but it is situated at Liberty road, Ibadan, Oyo State. She has staff strength of 12 members. Well is the main source of water used for processing and was pumped into the overhead storage tank, from where it circulate through the tap and is fetched uncovered buckets and plastic containers for use. The owner of the processing plants keeps snails. The plant is also a 3 bed room flat situated near a water canal and a disposal point. The finished products are packaged in take-away disposable packs and polyvinyl chloride (PVC) packs, sealed and stored in freezers. The finished products were sold in their shopping marts. Parts of the finished products were supplied in bulk in a larger coolers to major shopping marts, supermarkets and hotels in Ibadan and Lagos. The unsold product from these major shop dealers were returned back after a long period or if there were too many complains from the customers. These products were either re-processed and repackaged for another supply or discarded if it has deteriorated.

Processing plant C is owned by a former manager, who worked at processing plant A for many years before she established her own plant. It is situated at Ijora-Olopa, Lagos Island near a Lagoon,

Lagos State. The shops are close to NEPA building, and a busy market. Water for processing was obtained from uncovered wells in buckets and stored in open drums and a running tap drawn from the well. The finished products was sprayed in trays and stored at commercial cold room to be frozen, this was later weighed and packaged in different weights and stored in freezers and vended in the market near them. They also engaged in supplies to shopping marts and hotels in Lagos State.

Processing plant D is also situated at Liberty road, Ibadan, Oyo state. The shop is close to a busy road. They have their own commercial cold room. Water for processing was obtained from a covered well in buckets and stored in open drums. The finished products was sprayed in trays and stored at commercial cold room to be frozen, this was later weighed and packaged in cartoons and stored in cold room and vended to retailers in the markets.

Collection of samples

500 g of the samples were collected from raw (unprocessed) and after each step of the processing to packaging and the finished product for sales. Samples of water, raw materials and swabs of utensils used for processing and swabs of the working surfaces and freezers were also collected. Palms of the all processors/handlers were also swabbed. Forty samples were also purchased from vendors for microbiological analysis. All samples collected were held in ice pack and taken to the laboratory within 2 - 6 h of collection for analysis (Oranusi et al., 2003). Samples that could not be analyzed were stored at -4^{O}C in a freezer till the following day while seafood products were maintained at -20^{O}C till the following day.

Physico-chemical analyses

Temperature and pH of the water samples were measured at the point of collection using a digitron thermometer (model 275-K) as described by the methods of FAO (1997a,b) and standardized mercury in glass centigrade thermometer as described by Edema et al. (2001).

Isolation and Enumeration of isolates

Each PVC and disposable take-away packs of seafood products were cleaned externally with 70% ethanol to disinfect it. It was punched with a sterile forceps and100g was weighed into sterile wide-mouth beakers containing sterile distilled water. It was thoroughly shaken and an aliquot of 10ml was evacuated into sterile universal bottles. Also an aliquot of 100ml of the water samples was evacuated into sterile universal bottles. Appropriate serial dilutions of all the seafood and water samples were carried out and 0.2ml of the selected dilution was spread on duplicate plates using sterile glass spreader (Fawole and Oso 2001; Oranusi et al., 2003). This technique was used for the enumeration of total viable count, coliform, bacillus counts, staphylococcal counts*, Salmonella and Shigella* counts and fungi counts on plate count agar (Difco), eosin methylene blue (EMB) agar (Oxoid), Salmonella and Shigella agar (Oxoid), MacConkey agar (Oxoid), Mannitol salt agar (Oxoid), thiosulphate citrate bile salt (TCBS) agar and potato dextrose agar (Oxoid). All cultures were incubated at 37^{O}C for 24h except for coliform organism which was incubated at 37^{O}C and 44^{O}C. All the media used were weighed out and prepared according to the manufacturer's specification, with respect to the given instructions and directions. Samples of water, raw materials and swabs of utensils used for processing and swabs of the working surfaces and freezers, and Palms of the all processors/handlers were cultured for the presence of coliform organisms, *Vibrio* spp. and *Bacillus* spp. NAFDAC approved standard water was used as control.

Campylobacter *jejuni*

An aliquot of 1 ml was inoculated into Preston Enrichment Broth (SR0117; Oxoid). The contents were incubated at 42oC for 24 h (Scates et al., 2003). Two loopfuls of the broth were transferred onto Blood agar plates (Columbia blood + Lysed Horse Blood, Merck, Midrand, South Africa), wrapped in plastic pouches (AG0020C; Oxoid) and incubated at 42oC for 4 days under microaerophilic conditions using anaerobic jar (HP0031; Oxoid) containing Campgen sachets (CN0020C; Oxoid). Colonies appear ing round to irregular with smooth edges were presumed as *Campylobacter* (Lennette et al., 1985). A loopful of growth was placed in a drop of 3% hydrogen peroxide and appearance of bubbles was confirmed as positive for *Campylobacter* (Taulo et al., 2008).

Characterization of isolates

Confirmation of coliform organisms were carried out by inoculating colonies into lactose broth with Durham tubes and incubating at 37^{O} and 44 ^{O}C for 24 h and another 24h in the absence of gas production (Speck, 1976; Barker et al., 2001; Oranusi et al., 2003). The presence of gas constituted a presumptive test and the broth was streaked out on EMB agar incubated at 37 ^{O}C for 24h. Typical colonies on EMB plates appearing bluish black with greenish metallic sheen which are characteristics of E. coli or brownish colonies often convex and mucoid which are characteristics of Enterobacter aerogenes confirmed the presence of coliform organisms. Pure isolates of resulting growth were stored on nutrient agar slants at 4^{O}C for further confirmatory tests. The isolates were identified using morphological and biochemical methods as described by Jolt et al. (1994) which included IMVIC test, carbohydrate utilization, reaction on TSI, gelatin liquefaction, nitrate reduction, urease production and motility. Large, flat, irregular, wrinkled or smooth, ground-glass colonies, 4-6mm in diameter were counted as Bacillus. Confirmation was as described by Yusuf et al. (1992). Confirmation of typical colonies of S. aureus on Mannitol salt agar was on the basis of the results of catalase, coagulase, phosphatase production, nitrate reduction and carbohydrate utilization as described by Umoh et al. (1999). Isolation and confirmation of Salmonella and Shigella were according to the procedures recommended by Barker et al. 2001. The preenriched samples in lactose broth were subcultured into selenite F broth for selective enrichment, and on Salmonella-Shigella agar (SSA). Typical colonies were gram-stained and characterized (Barker et al., 2001; Oranusi et al., 2003). The sterility of each batch of test medium was confirmed by incubating one or two uninoculated tubes or plates along with the inoculated tests. The uninoculated tubes or plates were always examined to show no evidence of bacterial growth (Barker et al., 2001).

Haemolysis of human and sheep red blood cells

Isolated *S. aureus, S. epidermidis* and *Streptococcus faecalis* were inoculated on blood agar base (Oxoid) containing 10% sheep blood. The plates were incubated at 37^{O}C for 24 h and a zone of haemolysis around colonies was observed and reported as either alpha (α), beta (β), or gamma (γ) (Umoh et al., 1990; Barker et al., 2001; Oranusi et al., 2003).

Statistical analysis

One-way analysis of variance, and least significance difference (LSD) were used to compare means of fungi, total aerobic, staphylococcal, coliform and bacillus counts for bulk, cellophane and disposable take-away packaged seafood products using SPSS version 13.0 (SPSS Inc, 2002) and significant of differences tested

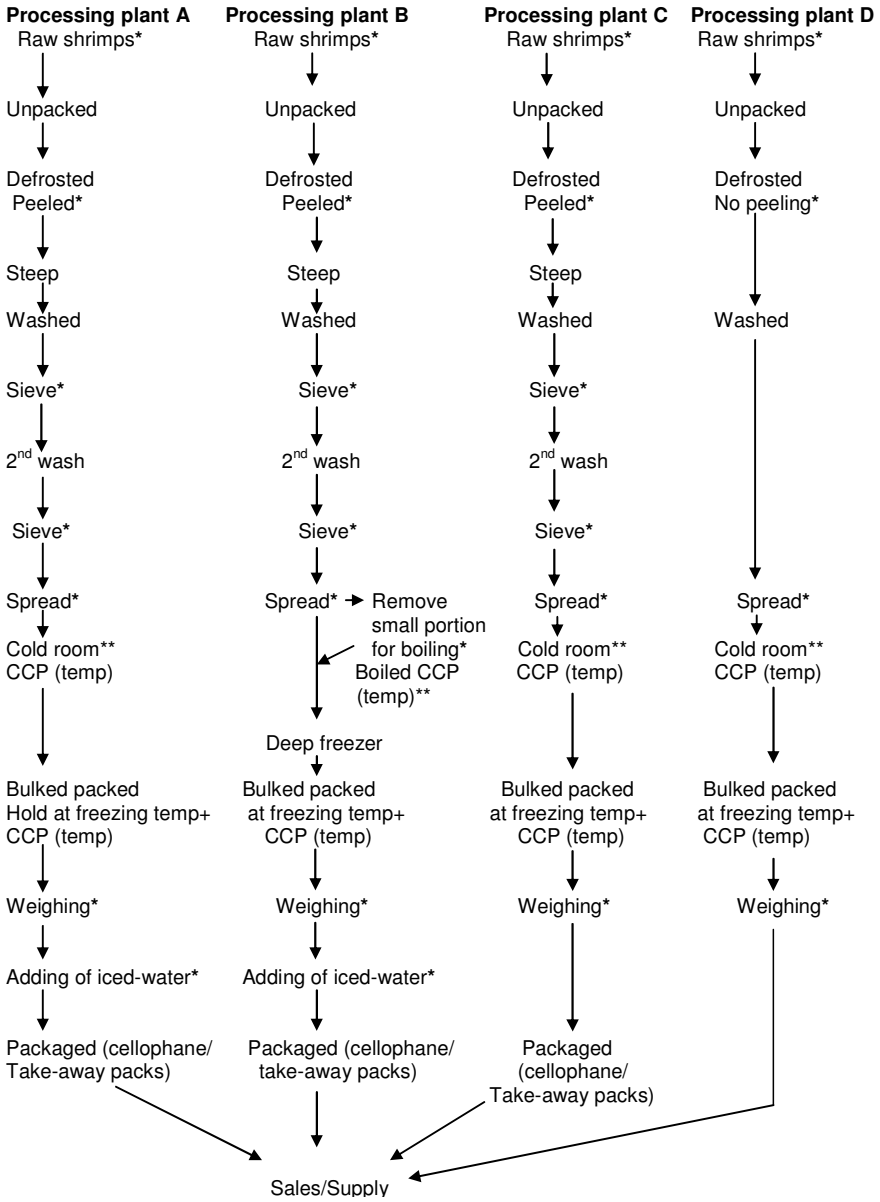

Figure 1. Processing and packaging of seafood products at processing plants A - D. Key: * = Hazard of contamination likely; ** = Hazards of survival; + = Hazards of microbial growth likely; CCP = critical control point.

using chi-square for categorical variables (Oranusi et al., 2003).

RESULTS

Flow charts showing hazards and CCP during processing and packaging of seafood products are shown in Figure 1. The flowcharts showed that Processing plant B boils some of their shrimps before packaging and uses deep freezer for drying and caking of the products, while A, C and D used cold room for drying and caking of the products. Processing plants A and B adds iced-water to the products during weighing to make it up to the desired weights before packaging. C and D do not add any form of water to their products during packaging.

All the four processing plants spread their products on open trays. Three processing plants (A-C) sieved their products before spreading on open trays. Three processing plants (A-C) soaked their products for sometime to allow the reddish colour of the shrimps and prawn to deepened. All the four processing plants also weigh their products into different quantities (Figure 1). Only processing plant B boiled part of her products, and have shopping mart and processing plants as well as engaged in supply to major supermarkets and shopping marts dealers. The physico-chemical properties of the water samples used by seafood processors/handlers are shown

Table 1. Physico-chemical properties of the water samples used in processing seafood products.

Sample	Colour	Odour	Taste	Presence of particles	pH	Temp°C
LA	Slightly turbid	Odourless	Offensive	Suspended solids	7.2	26.0
LB	Colourless	Odourless	Tasteless	None	8.0	27.5
IC	Colourless	Odourless	Tasteless	None	7.4	25.0
ID	Colourless	Odourless	Tasteless	None	6.8	25.2
LE	Colourless	Odourless	Tasteless	None	7.2	26.5
LF	Colourless	Odourless	Tasteless	None	6.5	25.2
LG	Slightly turbid	Odourless	Tasteless	Few particles	7.5	26.4
IH	Colourless	Odourless	Tasteless	None	7.4	25.0
II	Colourless	Odourless	Tasteless	None	6.8	26.2
IJ	Colourless	Odourless	Tasteless	None	7.8	28.5
Standard limit	Colourless	Not offensive	Not offensive	No visible solids	6.5 - 8.0	

in Table 1. Sample LA and LB are well water used by processing plant A. Sample IC and ID are most frequently used tap water and well water used by processing plant B. LE is NAFDAC approved sachet water used by Processing plant C. LF and LG are well water and Lagoon water samples used by processing plant C. IH is water sample from bore-hole storage tank used by Processing plant D. II and IJ are well water samples used by processing plant D. Some of the water samples, particularly the tap running from a bore-hole tank and the well water samples did not comply with the standard limits for drinking water. The pH ranged of 6.5 to 8.0 while temperature ranged from 25^{O}C to 28.5^{O}C (Table 1). The pH as recorded for the water samples and the pH for tap water and borehole water could be considered as being within acceptable range for natural waters. As far as the pH is concerned they vary from pH range of 6.8 – 7.5 indicating that sample LG had the highest pH value of 7.5.

Table 2 shows the microbial load, processing temperature and pH at different stages of seafood processing and packaging. The time-temperature exposure of seafood for the different processing plants gave an appreciable drop within processing hours for processing plant A having decreased from 30 to -8^{o}C; processing plant B having decreased from 29 to -8^{o}C; processing plant C having decreased from 29 to -8^{o}C and processing plant D having decreased from 28 to -8^{o}C. However, the temperature of the four products finally decreased to about 27 -28^{o}C during spreading (Table 2).

The time-pH exposure of seafood for the different processing plants gave an appreciable drop within processing hours for processing plant A having decreased from pH 6.8 to pH 6.5; processing plant B having decreased from pH 6.8 to pH 6.4; processing plant C having decreased from pH 6.8 to pH 6.5 and processing plant D having decreased from pH 6.8 to pH 6.0. However, the temperature of the four products from the 4 processing plants finally decreased to about pH 6.5 to pH 6.0 during spreading (Table 2).

All the samples taken before processing (Raw samples) had high count ranging from 5.60x10^{4} to 1.20x10^{5} CFU/ml for TVC; from 1.90x10^{4} to 3.50x10^{4} CFU/ml for CC; from 1.00x10^{4} to 3.60x10^{4} CFU/ml for SS; from 0.69x10^{4} to 5.80x10^{4} CFU/ml for SC; 0.41x10^{4} to 1.65x10^{4} CFU/ml for BC; and 0.70x10^{4} to 0.90x10^{4} CFU/ml for FC while samples taken during processing and when it has been packaged had high count ranging from 0.80x10^{4} to 1.50x10^{4} CFU/ml for TVC except fungi count (FC) which had low count ranged from 0 to 0.20x10^{4} CFU/ml at the point of sales and supply. Coliforms, *Micrococcus* sp. and *S. aureus* appeared in almost all the samples after peeling ranged from 1.10x10^{4} to 2.60x10^{4} CFU/ml and increased ranging from 0.80x10^{4} to 1.50x10^{4} CFU/ml for TVC at the point of sales and supply, while bacillus count increased after peeling ranging from 0.24x10^{2} to 1.04x10^{4} CFU/ml and 0 to 0.70 x 10^{4} at the point of sales (Table 2).

Table 3 shows the mean and range of microbial load of vended seafood product sold in Ibadan and Lagos, Nigeria. Total counts and most probable number (MPN) of coliform of raw and processed bulk, PVC, DTAP samples of vended seafood products sold in Ibadan and Lagos. Microbial load was different from one sample to the other. All exceeds standard limit 1.0 x 10^{2} CFU/ml (Table 3).

Out of 18 samples of vended processed and unprocessed shrimps purchased from different shops, 3 were PVC packaged processed shrimps and unprocessed shrimps, 3 were disposable take-away packaged processed shrimps and unprocessed shrimps, 3 were bulk-packaged unprocessed shrimps and processed shrimps (Table 3). While bulk-packaged processed and unprocessed shrimps had significantly ($P<0.05$) higher microbial loads, the PVC-packed and disposable take-away shrimps had higher coliform count. Processed shrimps had lower fungi counts (Table 3). Table 4 shows the frequency of occurrence of the organisms isolated and identified from the samples of water, raw materials and swabs of utensils used for plants. Samples E, F, G and H are

Table 2. Microbial loads, processing temperature and pH at various stages of processing and packaging seafood products for four processing plants.

Procedure/ period of sampling	Processing plant A mean			Processing plant B mean			Processing plant C mean			Processing plant D mean		
	Count CFU/g	T°C	pH	Count CFU/g	T°C	pH	Count CFU/g	T°C	pH	Count CFU/g	T°C	Ph
Raw												
TVC	6.40×10^4	30	6.8	5.60×10^4	29	6.8	1.20×10^5	29	6.7	1.16×10^5	28	6.8
CC	3.50×10^4			1.90×10^4			2.10×10^4			3.30×10^4		
SS	1.00×10^4			2.00×10^4			3.60×10^4			1.80×10^5		
SC	0.69×10^4			1.80×10^4			1.50×10^4			5.80×10^4		
BC	0.41×10^4			0.80×10^4			1.65×10^4			1.20×10^4		
FC	0.70×10^4			0.80×10^4			0.90×10^4			0.70×10^4		
After peeling												
TVC	1.36×10^4	29	6.8	1.10×10^4	29	6.5	1.50×10^4	28	6.5	2.60×10^4	28	6.8
CC	0.22×10^4			0.20×10^4			1.40×10^4			0.20×10^4		
SS	0.10×10^4			0.10×10^2			1.80×10^4			1.00×10^4		
SC	0.50×10^4			0.10×10^2			1.26×10^4			0.14×10^4		
BC	0.75×10^4			0.24×10^2			1.04×10^4			0.30×10^4		
FC	0.25×10^4			0.10×10^4			0.20×10^4			0.20×10^4		
After sieving												
TVC	1.30×10^4	29	6.8	2.60×10^4	29	6.8	1.00×10^4	28	6.8	4.40×10^4	27	6.5
CC	0.10×10^4			0.20×10^4			0.40×10^4			0.40×10^4		
SS	2.40×10^4			1.00×10^4			2.30×10^4			0.80×10^4		
SC	1.10×10^4			1.50×10^4			0.26×10^4			0.14×10^4		
BC	1.30×10^4			1.20×10^4			0.44×10^4			0.30×10^4		
FC	0			0.20×10^4			0.10×10^4			0.30×10^4		
After spreading												
TVC	2.60×10^4	29	6.5	0.70×10^4	28	6.4	4.40×10^4	27	6.5	2.60×10^4	28	6.0
CC	0.20×10^4			0.10×10^4			0.40×10^4			0.20×10^4		
SS	1.00×10^4			4.00×10^4			0.80×10^4			1.00×10^4		
SC	0.20×10^4			1.20×10^4			0.40×10^4			0.76×10^4		
BC	0.18×10^4			0.20×10^4			0.80×10^4			1.50×10^4		
FC	0			0			0.30×10^4			0.10×10^4		
Holding freezing temp												
TVC	1.00×10^4	-20	6.5	1.30×10^4	-20	6.5	0.70×10^4	-20	6.5	1.00×10^4	-20	6.4
CC	0.20×10^4			0.10×10^4			0.10×10^4			0.40×10^4		
SS	0.40×10^4			2.40×10^4			4.00×10^4			2.30×10^4		
SC	0.40×10^4			0.56×10^4			0.64×10^4			0.20×10^4		
BC	0.40×10^4			0.34×10^4			0.71×10^4			1.10×10^4		
FC	0			0			0			0		
Ready-to- sale/ supply												
TVC	0.80×10^4	-8	6.5	0.80×10^4	-8	6.4	1.30×10^4	-8	6.5	1.50×10^4	-8	6.5
CC	0.10×10^4			0			0.10×10^4			1.20×10^4		
SS	0.10×10^4			0			2.30×10^4			1.80×10^4		
SC	0.10×10^4			0.10×10^4			0.70×10^4			1.20×10^4		
BC	0.20×10^4			0			0.70×10^4			0.20×10^4		
FC	0			0			0.10×10^4			0.20×10^4		

Keys: Counts are means of triplicates samples; TVC = total viable count; CC = coliform count; SS = Salmonella-Shigella count; SC = staphylococcal count; BC = Bacillus count.

swab samples collected processing and swabs of the working surfaces and freezers, and palms of the all processors/handlers. Samples A-Z is swab samples of the utensils used by the different processing plants. Samples A, B, C and D are swab samples collected from freezer compartments used in storage of bulk raw products at the different processing from the freezers used sales outlets at the processing plants. Samples I, J, K and L are the swab samples of Scale pans used for weight measurement. Samples M, N, O and P are the swab samples of the processing and packaging tables of the different processing plants. Samples Q, R, S, and T are the swab samples of the water storage drum. Samples U, V, W and X are the swab samples of bowls used for fetching water, soaking and washing processed products. Samples Y, Z, a and b are swab samples of the sacks used in the storage of processed products at the four processing plants that were sampled.

Table 3. Mean and range of microbial load of vended seafood product sold in Ibadan and Lagos, Nigeria.

Procedures	Samples/counts CFU/g					
	Raw (unprocessed)			Processed		
	Bulk N = 3	PVC (n = 3)	DTAP (n = 3)	Bulk (n = 3)	PVC (n = 3)	DTAP (n = 3)
			Total viable count			
Mean	7.40×10^4	5.30×10^4	7.10×10^4	2.23×10^4	0.90×10^4	1.77×10^4
Range	4.60×10^4-1.20×10^5	3.90×10^4-6.40×10^4	3.90×10^4-1.16×10^5	1.00×10^4-4.40×10^4	0.80×10^4-1.10×10^4	1.20×10^4-2.60×10^4
			Coliform count			
Mean	2.03×10^4	2.90×10^4	2.33×10^4	0.10×10^4	0.30×10^4	5.33×10^4
Range	1.50×10^4-2.50×10^4	1.90×10^4-3.50×10^4	1.20×10^4-3.30×10^4	0-0.30×10^4	0.10×10^4-0.40×10^4	0.20×10^4-1.20×10^4
			Staphylococcal count			
Mean	1.90×10^4	1.65×10^4	2.02×10^4	0.34×10^4	0.30×10^4	0.56×10^4
Range	0.70×10^4-3.00×10^4	0.40×10^4-2.00×10^4	1.20×10^4-3.86×10^4	0.20×10^4-0.50×10^4	0.20×10^4-0.40×10^4	0.30×10^4-0.69×10^4
			Bacillus count			
Mean	2.20×10^4	1.39×10^4	2.00×10^4	6.93×10^3	1.07×10^4	1.37×10^4
Range	1.20×10^4-3.00×10^4	0.90×10^4-2.00×10^4	1.80×10^4-2.10×10^4	0.63×10^4-0.75×10^4	0.63×10^4-1.39×10^4	0.41×10^4-1.90×10^4
			Salmonella-Shigella count			
Mean	6.67×10^4	3.35×10^4	1.27×10^4	1.83×10^4	0.33×10^3	0.97×10^4
Range	1.00×10^4-1.80×10^5	1.65×10^4-4.80×10^4	0.80×10^4-2.00×10^4	0.80×10^4-2.40×10^4	0-0.10×10^4	0.10×10^4-1.80×10^4
			Fungi count			
Mean	8.33×10^3	1.20×10^4	1.33×10^4	0.67×10^3	0	0.10×10^3
Range	0.70×10^4-1.00×10^4	0.90×10^4-1.50×10^4	0.70×10^4-2.00×10^4	0-0.20×10^4	0	0-0.30×10^4

Keys: n = No. of samples tested, PVC = polyvinylchloride, DTAP= Disposable take-away packs.

Table 4. Frequency of occurrence of Isolates.

Isolates	Frequency
Bacterial	**No. (%)**
Achromobacterium sp	2 (1.2)
Bacillus cereus	24 (14.0)
Citrobacter	3 (1.8)
Enterobacter aerogenes	30(18.0)
Escherichia coli	15(8.8)
Flavobacterium sp.	19 (11.1)
Klebsiella sp	4 (2.3)
Micrococcus sp.	36(21.1)
Proteus sp	8 (4.7)
Pseudomonas sp.	3(1.8)
Salmonella sp.	7 (4.1)
Serratia sp	3(1.8)
Staphylococcus aureus	17(9.9)
Total	171(100.0)
Fungi	**No. (%)**
Aspergillus niger	2 (13.3)
Aspergillus formigatus	3 (20.0)
Fusarium sp	2 (13.3)
Mucor mucido	3 (20.0)
Neurospora crassa	2 (13.3)
Rhizopus sp.	3 (20.0)
Total	15 (100.0)

A total of 186 isolates were isolate and identified. One hundred and seventy-one (171) were bacterial isolates while fifteen (15) were fungi isolates (Table 4). The organisms obtained in this study include- *Micrococcus* sp. [36(21.1%)] and *Enterobacter* sp. [30(18.0%)] which were most frequently isolated from palm swab of the seafood processors/handlers and some of the utensils by these seafood processors/handlers. These pathogens were also present in all the palm swab of all the seafood processors/handlers, followed by *Bacillus* sp. [24 (14.0%)], *Flavobacterium* sp. [19 (11.1%)], *Staphylococcus* sp. [17(9.9%)], *Escherichia coli* [15(8.8%)], *Proteus sp* [8(4.7%)], *Salmonella* sp. [7(4.1%)], *Citrobacter sp* [3(1.8%)], *Klebsiella sp* [4(2.3%)], *Pseudomonas sp.* [3(1.8%)], *Serratia sp.* [3(1.8%)] and *Achromobacterium sp* [2(1.2%)] (Table 4). Ten of the 17 *S. aureus* isolates tested were of alpha haemolytic pattern and seven produced beta haemolysis (Table 4). The fungi isolates obtained in this study include *Aspergillus formigatus* [3 (20.0%)], *Aspergillus niger* [2 (13.3%)], *Fusarium sp* [2 (13.3%)], *Mucor mucido* [3 (20.0%)], *Neurospora crassa* [2 (13.3%)] and *Rhizopus* sp [3 (20.0%)] were obtained from the seafood handlers and utensils used at the four processing plants at any stage of the processing (Table 4).

Table 5 shows the distribution of isolates among seafood handlers at the four processing plants sampled. Forty-seven (27.5%) of the isolates and 8 (46.7%) of the fungi isolates were obtained from the handlers working at

Table 5. Distribution of Isolates among seafood handlers in the processing plants

Isolates	Frequency	Seafood handlers														
Bacterial	**No. (%)**	**HA**	**HB**	**HC**	**HD**	**HE**	**HF**	**HG**	**HH**	**HI**	**HJ**	**HK**	**HL**	**HM**	**HN**	**HO**
Achromobacterium sp.	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bacillus cereus	7	-	-	-	+	-	+	+	+	+	-	+	-	+	-	-
Citrobacter sp.	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Enterobacter aerogenes	13	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+
Escherichia coli	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Flavobacterium sp.	5	-	-	+	-	-	-	-	+	-	+	-	+	-	-	+
Klebsiella sp.	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Micrococcus sp.	13	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+
Proteus sp.	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas sp.	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Salmonella sp.	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Serratia sp.	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Staphylococcus aureus	8	+	-	-	+	+	-	+	-	-	+	-	+	-	+	+
Total	47 (27.5)	3	0	3	3	3	2	4	4	3	4	3	3	3	3	5
Fungi	**No. (%)**															
Aspergillus niger	1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Aspergillus formigatus	1	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Fusarium sp	1	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Mucor mucido	1	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Neurospora crassa	1	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Rhizopus sp.	2	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-
Total	7 (46.7)	0	0	1	0	0	0	2	0	2	0	0	0	0	2	0

the four processing plants sampled. Only *Bacillus cereus* [7 (4.1%)], *Enterobacter aerogenes* [13 (7.6%)], *E. coli* [1 (0.6%)], *Flavobacterium* sp. [5 (2.9%)], *Micrococcus* sp. [13 (7.6)], and *S. aureus* [8 (4.7%)] as well as *A. niger* [1 (6.7%)], *A. formigatus* [1 (6.7%)], *Fusarium sp* [1 (6.7%)], *M. mucido* [1 (6.7%)], *N. crassa* [1 (6.7%)] and *Rhizopus* sp [2 (13.3%)] were obtained from the seafood handlers working at the four processing plants at any stage of the processing (Table 5). Possible sources of contamination common to the four processing plants include the contamination from handlers (Table 5). No *Achromobacterium sp*, *Citrobacter sp, Klebsiella sp*, *Proteus sp*, *Pseudomonas sp., Salmonella* sp., *Serratia sp.,* Vibiro sp. isolates or human enteric viruses were isolated from the seafood handlers working at the four processing plants at any stage of the processing (Table 5).

Distribution of isolates among the utensils used at the four processing plants sampled is shown in Table 6. A total of 123 (71.9%) of the bacterial isolates and 8 (53.3%) of the fungi isolates were obtained from the swab samples of all the utensils used at four processing plants sampled (Table 6). Only *Achromobacterium sp* [2 (1.2%)], *Bacillus cereus* [17 (9.9%)], *Citrobacter sp* [3 (1.8%)], *Enterobacter aerogenes* [17 (9.9%)], *E. coli* [14 (8.2%)], *Flavobacterium* sp. [14 (8.2%)], *Klebsiella sp* [4 (2.3%)], *Micrococcus* sp. [22 (12.9)], *Proteus sp* [8 (4.7%)], *Pseudomonas sp.* [3 (1.8%)], *Salmonella* sp. [7 (4.1%)], *Serratia sp.* [3 (1.8%)] and *S. aureus* [9 (5.5%)] as well as *A. niger* [1 (6.7%)], *A. formigatus* [2 (13.3%)], *Fusarium sp* [1 (6.7%)], *M. mucido* [2 (13.3%)], *N. crassa* [1 (6.7%)] and *Rhizopus* sp [1 (6.7%)] were obtained from the seafood handlers working at the four processing plants at any stage of the processing (Table 6). Possible sources of contamination common to the four processing plants include the presence of water canal near the house and near the wells, water, water vessels, raw materials, utensils and environments. Additional source includes presence of automobile mechanic garage, disposal points and snail in or near the processing plant B and D. No Vibiro isolate or human enteric viruses were isolated from products of the four processing plants at any stage of the processing (Table 6).

DISCUSSION

The results of this study have shown that there was faecal contamination of most of the vended seafood products, utensils and palms of the seafood handlers, and water from both protected and unprotected water sources. This was illustrated by the presence of the indicator organisms. Incidences of *E. coli, Enterobacter aerogenes* and other index of poor sanitary quality found in this study are in agreement with those of Trevett et al. (2005) and Hogue et al. (2006) who found *E. coli* in 29%

Table 6. Frequency of occurrence of Isolates by Utensils used by the Processing plants

Isolates	Frequency	Samples (Utensils)																												
Bacterial	**No. (%)**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**	**T**	**U**	**V**	**W**	**X**	**Y**	**Z**	**a**	**b**
Achromobacterium sp	2	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Bacillus cereus	17	+	+	+	-	-	-	-	-	+	+	-	+	-	+	+	-	-	+	+		+	+	+	-	+	+	+	+
Citrobacter	3	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+
Enterobacter aerogenes	17	-	+	+	-	-	-	+	-	-	+	+	+	+	-	+	+	+	+	-	-	+	+	+	+	-	-	+	+
Escherichia coli	14	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	-	+	+	+	-	+	+	-	-	+	+	+	+
Flavobacterium sp.	14	+	+	-	-	-	-	-	+	-	+	+	-	+	-	-	+	+	-	-	-	+	-	+	+	+	-	+	+
Klebsiella sp	4	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
Micrococcus sp.	22	-	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+
Proteus sp	8	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	+	+	-	+	-	-	-	-	-	+	+
Pseudomonas sp.	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+
Salmonella sp.	7	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	+	-	-	-	-	+	+	+
Serratia sp	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+
Staphylococcus aureus	9	+	-	+	-	-	-	-	+	+	-	-	-	+	-	-	-	-	+	-	-	-	-	-	+	-	-	+	+
Total	123(71.9)	3	4	4	0	0	0	2	4	3	5	4	6	6	4	6	3	5	6	4	0	7	4	5	4	5	5	12	12
Fungi	No. (%)																												
Aspergillus niger	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Aspergillus formigatus	2	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Fusarium sp	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Mucor mucido	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-
Neurospora crassa	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Rhizopus sp.	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Total	8 (53.3)	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	3	2

of stored water and borehole water samples, respectively. *E. coli* is an indicator of faecal contamination and faecal contamination is associated with poor environmental sanitation (Trevett et al., 2005). The high incidence of *E. coli* (13%) in boreholes is a concern as such sources are usually regarded as “safe” (Taulo et al., 2008).

The areas under study have disposal points/refuge dumps, characterized with water lodge, water canals, Lagoon, and litters of illicit defeacation that are usually carried during the rainy season. Leaching of refuge dumps and litters of faeces contents and flooding of human and animal wastes into the wells during rainy season could be other possible sources of contamination in the wells and boreholes (Mathess et al., 1988; Taulo et al., 2008). Individual water drawing containers, (especially those with ropes) practiced by most households were also prone to contamination of water (Taulo et al., 2008). In this regard, considering the associated risks, it is strongly recommend that attention be focused on ensuring a supply of biologically safe water for drinking or processing seafood products and improving its management from the source to the storage point.

For boiled shrimps packaged in processing plant B, heat treatment of food generally (e.g. cooking) not only improves the taste, smell, appearance and digestibility, it also reduces the number of microorganisms, improves keeping qualities by inhibiting moulds, yeast and bacteria that promote decay and infection. Thus, heat treatment is a practice aimed at improving the overall safety of food. This makes it a CCP (Oranusi et al., 2003). Boiled shrimps prepared by the processing plant B attained a temperature of 60 ^{O}C immediately after colour change and that temperature should be high enough to kill large numbers of vegetative cells, but not heat-resistant spores (Bryan, 1988; Oranusi et al., 2003). The total viable count of recorded immediately after colour change from processing plant B could be explained either by survival of spores which could have come initially from the unprocessed (raw) samples or by reduction of, but not total elimination of, a very large number of vegetative cells that propagated during steeping (Obuekew and Ogbimi, 1989; Inaebo et al., 2000; Oranusi et al., 2003). Studies in these environments also confirmed the presence of high levels of spores and vegetative cells in shrimps.

The practice of sieving and spreading of the processed products appears the likely point for the contamination with coliform, *Bacillus* and *Staphylococcus.* Frozen in cold room/ deep freezer at freezing temperature overnight and holding at freezing temperature for storage and sales appear to be the major CCP of seafood products. Decreases in total viable count, coliform count, staphy-

lococcal count, *Salmonella* and *Shigella* count, and bacillus count were observed as processing progresses, indicating that these microorganisms were heavily present before processing. During the interval of interval of holding, spores that survived the freezing temperature, could germinate and injured vegetative cells could resuscitate. The high counts found in some of the products at the point of sales/supplies for products from processing plants A and B could be associated with the processing method adopted since these processors/handlers sieved their products after washing as opposed to processing plant D who do not sieved before spreading. Sieving before spreading could have reduced contamination by pathogenic microbes that may reach the product during and after sieving.

The isolation of *E. coli, Enterobacter aerogenes*, *S. auerus* and *Bacillus cereus* from seafood products is attributed to post-processing contamination from processors/handlers, water used for washing and water used in making up the weight of the products, utensils and animals present in the environment. However, the high pH (6.5-8.0) recorded for the water samples may have contributed to keeping the count of pathogens high in processed seafood products. The freezing temperature of -8 to -20^{o}C and high water activity (a_w) which is optimum for growth of these pathogens contributed to the high microbial load reported in this study. This finding differed from the reports of Oranusi et al (2003) in a similar study on HACCP of kunu.

The rapid defrosting of products observed in the product from processing plant B could be attributed to the fact that the products was caked in deep freezer and power failure during freezing as opposed to processing plants A, C, and D in which the products are caked in cold room. However, the improper freezing due to use of freezer instead of cold room for freezing and lack of constant power supply during processing and packaging supported growth of pathogens in products from processing plant B, thereby aiding quick spoilage of the products.

In this present study, almost all swab samples of the seafood handlers palm haboured *Micrococcus* sp., *Enterobacter* sp., *Bacillus* sp. and *Staphylococcus aureus,* while prominent microorganisms variously haboured include *Micrococcus* sp., *Enterobacter* sp., *Bacillus* sp., *Flavobacterium* sp., *Staphylococcus* sp., *Escherichia coli*, *Proteus sp*, *Salmonella* sp., *Citrobacter sp*, *Klebsiella sp*, *Pseudomonas sp., Serratia sp.* and *Achromobacterium sp*., *Aspergillus formigatus*, *Aspergillus niger*, *Fusarium sp*, *Mucor mucido*, *Neurospora crassa* and *Rhizopus* sp. Improper and faulty processing and handling by seafood processors/handlers could be sources of microbial chance inoculation, microbial food poison, food intoxication and food spoilage hence, processors/handlers may be counter productive by being responsible for public health hazard and loss of revenue (Bankole et al., 2005).

The pathogens isolated in this present study are similar to the microorganisms reported by Bankole et al. (2004; 2005) in a similar study where all the palms haboured *Staphylococcus aureus* and the palms of hotel operators among the food vendors sampled were reported to have harboured the least types of microorganisms. Olawale et al. (2005) reported nine bacterial genera and two fungi in a similar study which include *S. auerus*, *E. aerogenes*, *Str. faecalis*, *E. coli*, among other organisms (Okonko et al., 2008a,b,c).

The presence of *Bacillus cereus* and *E. coli* reported in this study is also in agreement with the findings of Adesokan et al. (2005) who reported the presence of *Bacillus sp* and *E. coli* among other organisms. All these pathogen isolated in this study are of food and public health implication and hence, hazardous and injurious to human health if consumed. There were significant correlation between bacteriological quality, food hygiene training and waste management polices of most processing plants studied.

The HACCP and microbiology quality of seafood as affected by processors/handlers' hygiene suggests that there is need to improve on hygienic and sanitary practices in public frozen seafood processing outlets in order to obtain relatively safe products for consumption. The new approach to supervision of seafood quality as affected by processors'/handlers' hygiene, the HACCP system works rationally as it is based on analysis of systematically assembled data on the causes and conditions which evoked the illness of the consumers by seafood products or meals.

The isolation of *Staphylococcus aureus* and *Salmonella* sp. in this study is of practical impact. It shows that most of the seafood products might have been contaminated from source. It is an evidence of poor sanitary conditions and lack of or inadequate potable water. *Salmonella* species such as *Salmonella typhi* is a bacterium that causes typhoid fever (enteric fever), an acute, life-threatening febrile illness (CDC, 2008). The disease is a cause for concern and a major public health problem in developing countries (Asia, Africa), especially in Nigeria due to poor sanitary conditions and lack of or inadequate potable water (Ibekwe et al., 2008). It is mainly transmitted through food or drink or water, contaminated with urine or faeces of infected people or a chronic carrier (Utah, 2005; CDC, 2008; Ibekwe et al., 2008). Travelers who are visiting relatives or friends and who may be less likely to eat only safe foods (cooked and served hot) and beverages (carbonated beverages or those made from water that has been boiled) are at greater risk (Steinberg et al., 2004; Ibekwe et al., 2008).

The presence of *Staphylococcus aureus* and *Salmonella* sp. was also reported in previous studies on ready-to-eat seafood by Okonko et al. (2008b, c) and in sausages sold in Abeokuta and Benin-city, Nigeria in a study by Oluwafemi and Simisaye (2005). According to Oluwafemi and Simisaye (2005) most of the sausage being sold as ready-to-food pose health risk to consumers, making it

imperative to institute not only sanitary measures during its production and sales bit for retailers selling raw of pre-processed foods to have a steady source of power supply.

The observed pattern of low incidences of *C. jejuni* was consistent with earlier reports on water contamination (Botton et al., 1987). According to Botton et al. (1987) *C. jejuni* is very difficult to isolate and is usually detected in small numbers. The presence of *E. coli* in both seafood product and samples of water used in processing the products demonstrates a potential health risk as the organism is pathogenic and causes complications in children (Taulo et al., 2008).

Salmonella contamination is usually associated with contaminated food and animal feeds and its presence in this study signals faecal contamination of both human and animal origin (Dondero, 1977). In this study, *Salmonella* was detected in 24% of the water samples and the seafood products; this finding is supported by that of Dondero et al. (1977) and Phan et al. (2003). Contamination of *Salmonella* at the source was observed to be higher in samples that were collected from unprotected sources and possibly reflects exposure of the water to animals. It was alarming to observe people abstracting water from wells close to Lagoon bed sand (at a depth of less than 30 cm), sources that are associated with *Salmonella* contamination and other pathogenic micro organisms such as *Vibrio cholera* (Taulo et al., 2008).

Most of the organisms found in this study are those commonly found in soil and water. But the presence of other indicator organisms like *E. coli* and *Enterobacter aerogenes* in those water samples might be the result of possible contamination during sales or unhygienic handling of seafood right from the processing plants. The presence of the most frequently isolated index of water quality and indicators of faecal contamination such as *Escherchia coli* and Enterobacter aerogenes reported in this study is an indication of faecal contamination of the water used for processing frozen seafood products as a result of possible burst along pipe lines or unhygienic handling of the water right from the treatment plant for tap water and borehole water (Edema et al., 2001; Okonko et al., 2008a,b,c) or contamination of the seafood products itself during processing or directly from source and this might have adverse effect on the health of the consumers (Adebolu and Ifesan, 2001; Okonko et al., 2008b,c).

The presence of *Staphylococcus auerus*, a pathogenic organism of public health concern and significance in these frozen seafood products might have contaminated the processed frozen seafood products from source as a result of handling by processors. Improper handling and improper hygiene might lead to the contamination of ready-to-eat food and this might eventually affects the health of the consumers (Dunn et al., 1995; Adebolu and Ifesan, 2001, Omemu and Bankole, 2005; Okonko et al., 2008b,c). It is therefore suggested that frozen seafood processors should be educated on the adverse effect of using untreated or polluted water for processing as these could serve as sources of faecal contamination. However, the processors/handlers should observe strict hygienic measures so that they will not serve as source of chance inoculation of microorganisms and contami-nation of these processed frozen seafood products.

The Standard Organization of Nigeria (1985) stated that coliform bacteria and pathogenic microorganisms should not be present in beverages. This applies also to other food products. It was reported that counts of 10^7 cells/g for *B. cereus* (ICMSF, 1974), and 10^6 cells/g for enterotoxigenic *S. aureus* (Bergdoll, 1979) are required to present a risk of intoxication. It is therefore, important to note that holding of seafood products at temperature below optimal freezing temperature for sales/supply could be risky as this support the growth of most mesophile.

The vended shrimps had counts ranging from 10^2-10^4 cells/g for coliforms and *S. aureus* and 10^1-10^6 cells/g for *B. cereus*. Though processing, packaging and storage could be used to improve the hygienic quality of seafood products, inadequate application of the processes and faulty practices may negate their benefits (Ehiri et al., 2001; Oranusi et al., 2003). Such practices as peeling, washing, sieving, spreading, freezing, packaging, and storage in a contaminated environment may lead to post-processing contamination. Holding the products for sales/supply at defrosted temperature and freeze-thawed products could encourage growth of these pathogens especially *Bacillus spp*. to hazardous levels. In the event of starter failure for naturally fermented product and under appropriate temperature emetic and diarrhea-genic toxin could be elaborated especially when the product is held at ambient temperature for processing and sales (Bryan et al., 1991; Oranusi et al., 2003).

Therefore, the major hazards associated with processing of seafood products are, the presence of spores of pathogenic strains which could germinate at ambient temperature after a freezing shock from caked products. The 10^4 cells for *B. cereus* appear safe, but inadequate drop in pH and holding temperature for sale may encourage growth to hazardous levels (Bryan et al., 1991; Oranusi et al., 2003). The presence of coliform and *S. aureus* and processing and packaging in a contaminated environment could present a risk (Okonko et al., 2008 a,b,c). More so, the *S. aureus* isolates were alpha haemolytic and likely to be human botypes and more enterotoxigenic than animal biotypes which are often beta haemolytic (Bergdoll, 1979; Oranusi et al., 2003). A study of complimentary food preparation and handling in Eastern and Northern Nigeria also confirmed the presence of enteric pathogens and spores of pathogens (Ehiri et al., 2001; Oranusi et al., 2003).

The presence of indicator and other organisms examined in this study is of special concern and perhaps the greatest danger associated with water used for food processing, drinking purposes and for human consumption is

contamination by human excrement (Edema et al., 2001; Okonko et al., 2008a,b,c). The need for microbial assessment of water for production of seafood and food drinks should also be emphasized to reduce possible contamination (Fagade et al., 2005; Okonko et al., 2008b,c).

The higher microbial loads in the stored water samples compared to the source water samples possibly demonstrates a wide variation of poor hygiene practices in the processing plants. This is supported by the observed practices and their association with high microbial loads. Attachment of microorganisms on the surfaces of the working benches, surface walls of utensils and water storage containers and eventual contamination of the water and the products is likely to have occurred (Roberts et al., 2001; Osmundsen, 2005; Taulo et al., 2008). Water fetched from wells and taps were transferred into containers, facilities that are not washed for several days, leaving sediments to settle at the bottom of the containers (Lindskog and Lindskog, 1988). These sediments which are mostly organic in nature, serve as nutrients for pathogens for their growth (Momba and Kaleni, 2001; Luby et al., 1999; Taulo et al., 2008).

This current findings with the unsafe water used for processing seafood products and poor sanitary conditions of the environment where these seafood products are processed and the lack of proper personal hygiene of the processors/ handlers working at the different processing plants and the vendors are grim reminders of the need to address water and sanitation urgently in these environment following findings. The study has also demonstrated that water used for both drinking, cooking and processing seafood products in the areas under study is of poor quality (microbiologically) and the contamination is possibly due to poor management of water and existence of poor sanitation (Taulo et al., 2008). The presence of *E. coli* in borehole water is of public significance as it is indicative of faecal contamination. Considering that fingers are prone to faecal contamination during toilet use (Shojaei et al., 2005), such practices of peeling shrimps with unwashed hands can easily promote occurrence of diarrhoeal disease outbreaks through cross-contamination. In these processing plants, implementation of interventions requires a careful consideration of habits and local culture of the handlers.

In conclusion, the HACCP and microbiological quality of seafood products as affected by processor/handlers' hygiene revealed that the contamination of processed seafood products is multifactorial and many factor contributed to the contamination including handlers unhygienic conditions, dirty environment and poor quality of water (Oranusi et al., 2003; Okonko et al., 2008a,b,c). The CCP for seafood products are peeling, washing, sieving, spreading, freezing and packaging after processing and holding at freezing temperature for sale.

From the findings of this study, it is therefore necessary to recommend that public awareness programmes should be employed to educate owners of seafood processing plants, food processors, food vendors and general populace on the need for food safety and the requirement for water used for human consumption. Water should be adequately treated before use and NAFDAC should ensure and enforce strict compliance of the recommended water and processed food standards as regards the production and sales of processed and packaged seafood products. Water to be used for processing food purposes should be boiled and filtered where necessary before use in processing read-to-eat seafood products for human consumption. It is therefore suggested that frozen seafood processing operators should be educated on the adverse effect of lack of proper personal and environmental hygiene and sanitation, and using untreated or polluted water for processing as these could serve as sources of faecal contamination. There is also the need to educate the owners of processing plants on the hazards and CCPs of processing and packaging seafood products. Such control measures and monitoring procedures as washing hands at intervals with antiseptic soap/detergents during peeling, processing and packaging, checking indicator of heat treatment by use of colour change as in the case of boiled products, washing raw (unprocessed) products before sieving, washing equipment and utensils thoroughly with detergents before and after use, sieving before spreading, using boiled and cooled water for washing and packaging products in qualities that could be sold off the same day especially where there is no cold rooms and stand-by alternative power supply are necessary for processing and packaging a safe seafood product of high microbiological quality and at zero hazardous tolerance.

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